A virtual cohort study of SARS-CoV-2 infection and Covid-2 vaccination

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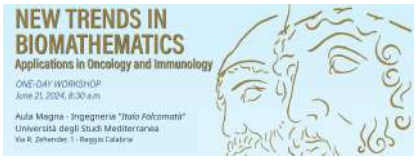


aim

- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has a variable clinical presentation.
  - Most individuals present with a very mild disease, often asymptomatic, and a few develop a life-threatening disease requiring intensive care.
  - The mortality rates also differ across the globe, ranging from 0.5-13%.
  - The strongest determinant of disease severity is age, with children presenting almost exclusively with mild disease (Brodin, 2020), while the elderly, over 70 years of age are much more likely to develop severe COVID-19.
- This variation is likely due to both host and pathogen factors
  - Host factors may include differences in the immune response due to genes and immunological history.
  - Pathogen factors include transmission, entry and spread within the host (load), tropism (spike-ACE2 affinity), virus virulence (replication speed) and disease mechanisms.

aim

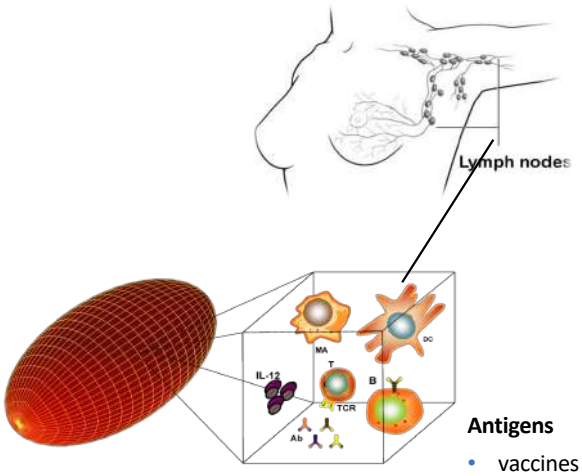
Understand how

- the magnitude of viral growth, and
- the subsequent innate and adaptive response required to achieve control of infection

impact in the differences observed in the immune response to SARS-CoV-2,

- We used computer simulation to create a **virtual cohort** of infected individuals to study the effects with respect to both host and pathogen factors

a computational (agent-based) stochastic model



Antigens

- vaccines
- virus
- bacteria
- cancer cells
- ...

Cells

- B-cell
  - B-1
  - B-2
  - PLB
- CD4 T-cell
  - Th1
  - Th2
  - Th17
  - Treg
- CD8 T-cell
- NK
- Mφ
  - M1
  - M2

DC

EP (virus target)

Immunoglobulins

- IgM
- IgG
  - IgG1
  - IgG2
- IC

Interleukins/cytokines

- Danger
- IL-2
- IL-12
- IFN-γ
- IL-4
- TNF-α
- TGF-β
- IL-6
- IL-10
- IL-18
- IL-23
- IFN-β
- IL-1β

immunological features

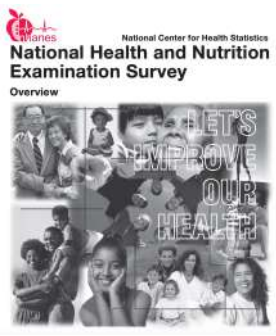
- Innate + Adaptive immunity
- Humoral + Cellular immunity
- Clonal selection theory (random generation of TCRs and BCRs)
- Clonal deletion theory (positive/negative selection of T-cells in the thymus)
- Immunological memory
- Homeostasis
- Affinity maturation and hypermutation
- Peptide digestion and presentation (class-1, class-2)
- Matzinger's danger signal theory
- T-cells replicative senescence (the Hayflick limit)
- Cytokine activation/inhibition of immune cells' functions
- T-cell anergy (lack of second signal)
- B-cell anergy (overstimulation)
- Peripheral tolerance by Tregs
- Attrition

events

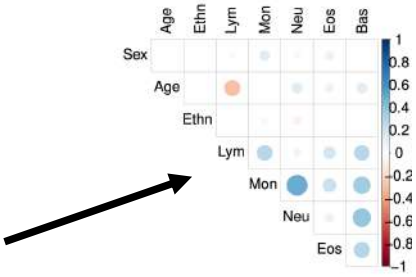
1. Infection: An infection dose $V(0) = V_0$ is injected into the simulated volume
2. Endocytosis: the virus enters epithelial cells (EP)
3. Biosynthesis: the viral RNA and viral proteins are made and assembled into new virions that are released by budding (exocytosis) from infected cells (SARS-CoV-2 follows a *lysogenic cycle*, that is, it does not kill the host). At this stage, infected/injured EP
   - DAMPs release: release danger signal (D) (generally indicating interferon, cytokines, DAMPs = damage associated molecular patterns)
   - Inflammation: release IL-6
   - Endocytic presentation: process the viral proteins leading to their presentation on class I HLA molecules
4. B phagocytosis: B cells phagocyte, internalise, process and present viral peptides on class II HLA
5. Response to Danger:
   - NK response: Natural killer cells (NKs) release IFNg upon bystander stimulation by danger
   - M response: Macrophages (M) respond to danger (e.g., DAMPs) via TLR4 releasing TNFa and IL-6
6. M activation: macrophages become activated by IFNg (activated M have a greater phagocytic activity)
7. Active M
   - M phagocytosis: M internalise, process and present viral peptides on class II HLA; in presence of IFNg they release IL-12; they also release TNFa
   - DC activation: M release TNFa which activate dendritic cells (DC)
8. DC phagocytosis & endocytosys: DC phagocyte, internalise, process and present viral peptides on class II HLA (exocytic pathway) but also on class I HLA (endocytic pathway)
9. Th activation: in presence of danger signal, resting T helper lymphocytes are activated by interaction with peptide-bound HLAs on professional antigen presenting cells (M and DC, mainly DC) surface by means of specific interaction with their T-cell receptors (TCR); if no danger is present, the Th cells becomes anergic upon interaction of its TCR with the HLApepide complex
10. Th stimulation by APCs: activated Th interacting with antigen presenting cells (M, DC)
    - Th duplication: start clone expansion; part of the clones become memory cells
    - Th cells release IL-2
    - M release IL-6
    - Th1 release IFNg
    - Th2 release IL-4
    - release IL-12 in presence of high local concentration of IFNg
    - Treg release TGFb and IL-10
11. Th stimulation by B: activated Th interacting with B cells
    - B duplication: stimulate B cells to start clone expansion; part of the clone become memory
    - Th duplication: start clone expansion; part of the clones become memory cells
    - release IL-2, IL-12
    - Th1 release IFNg
    - Th2 release IL-4
    - Treg release TGFb and IL-10
12. Th differentiation: depending on the local concentration of IFNg, IL-10, IL-4, IL-6, IFNb, IL-12, IL-18, IL-2, TGFb and IL23, active T helper cells undergo class switch into Th1 and Th2
13. B differentiation: B cells differentiate to antibody-secreting plasma B cells (PLB);
14. Isotype switch: B cells perform immunoglobulin class switching, that is, change production of immunoglobulin from the isotype IgM to the isotype IgG
15. Antibodies production: Plasma cells secrete antibodies
16. Humoral response: antibodies inhibit viral particles by opsonization; the result are the immuno-complexes that are eventually cleared by macrophages
17. Tc activation: in presence of IL-2, resting cytotoxic T cells (Tc) are activated by the interaction of their TCR with DC presenting on class I HLA the viral peptides but only in presence of IL-2
18. Tc duplication: activated Tc interact with infected EP cells presenting viral peptides on class I HLA molecule
    - Cytotoxic response: kill infected EP (this will further release danger signal)
    - Tc start duplication

Virtual Cohort

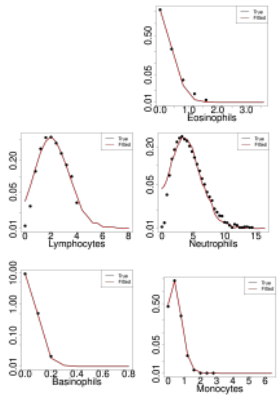
After the pre-processing phase, the dataset was reduced to a sample of 12150 individuals defined by the following characteristics: **sex, age, ethnicity, and WBC (i.e., lymphocytes, monocytes, neutrophils, eosinophils, and basophils).**



NHANES (2017-2020)

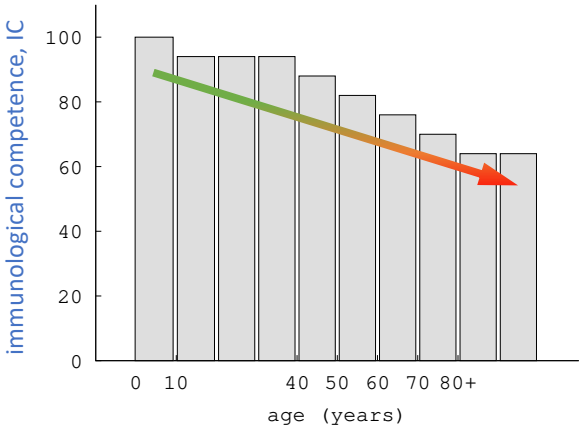


- Cell counts are correlated ==> Multivariate distribution
- Age are inversely correlated to Lymphocytes (and others)



host factors

We define the immunological competence, IC (can also be called immuno-senescence[1] factor, is the dual concept of immune deficiency)



$$IC = -0.0045\ age + 1$$
$$0 < IC \leq 1$$

[1] Aiello Anna, et al. (2019) Immunosenescence and Its Hallmarks. Front Immunol, 10:2247
https://www.frontiersin.org/article/10.3389/fimmu.2019.02247

host factors: innate immuno-senescence

- Phagocytic activity of Antigen Presenting Cells (i.e., Macrophages, Dendritic cells)

$$p_M = IC \cdot u \qquad u \sim U_{[a,b]} \qquad a = 400^{-1}, b = 10^{-2}$$

$$p_{DC} = IC \cdot v \qquad v \sim U_{[c,d]} \qquad c = 5a, d = 5b$$

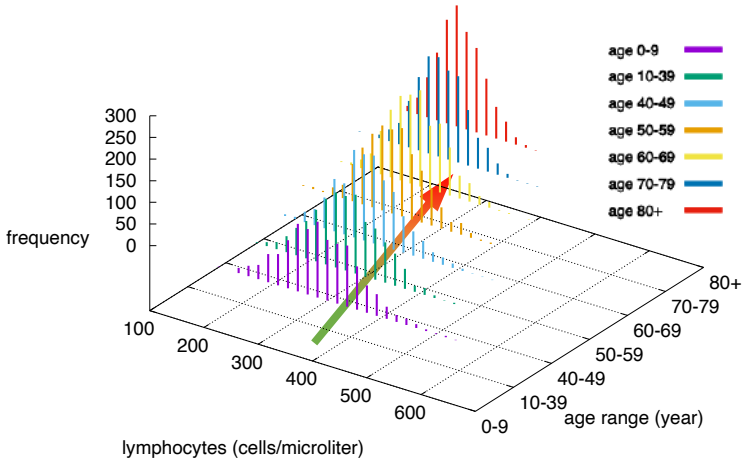
host factors: adaptive immuno-senescence

- Reduced leukocyte counts

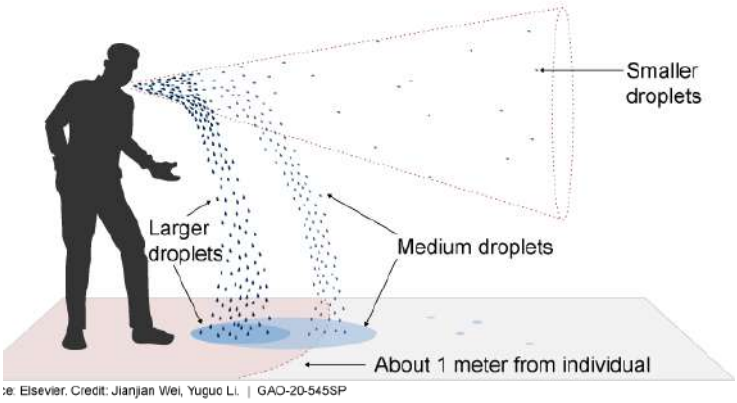
Normal Reference Range

- White blood cell count 4.0–11.0 x 10^9 /l
- Differential white cell count
  - Neutrophils 2.0–7.0 x 10^9 /l (40–80%)
  - Lymphocytes 1.0–3.0 x 10^9 /l (20–40%)
  - Monocytes 0.2–1.0 x 10^9 /l (2–10%)
  - Eosinophils 0.02–0.5 x 10^9 /l (1–6%)
  - Basophils 0.02–0.1 x 10^9 /l (<1–2%)

Lymphocytes $\sim N(\mu, \sigma) \cdot IC$



pathogen (virus) factors



- The viral load infecting an individual is diverse.

$$V(0) = V_0 = u \qquad u \sim U_{[a,b]}$$

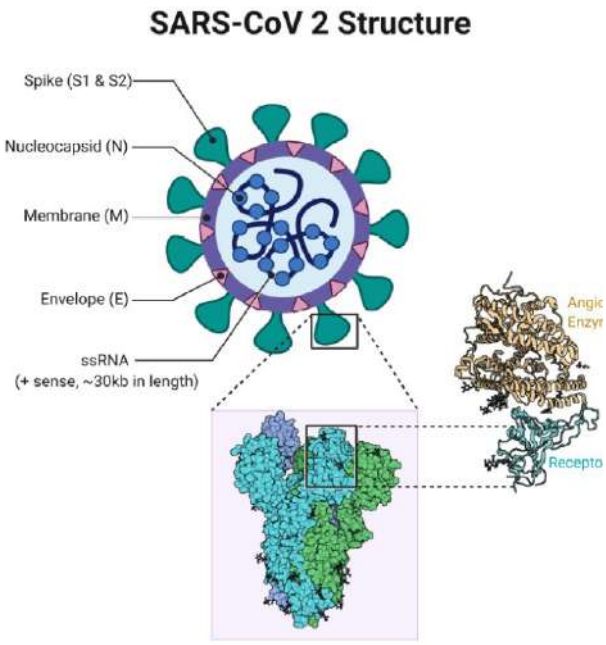
$$a = 5, b = 5 \cdot 10^5$$

pathogen (virus) factors

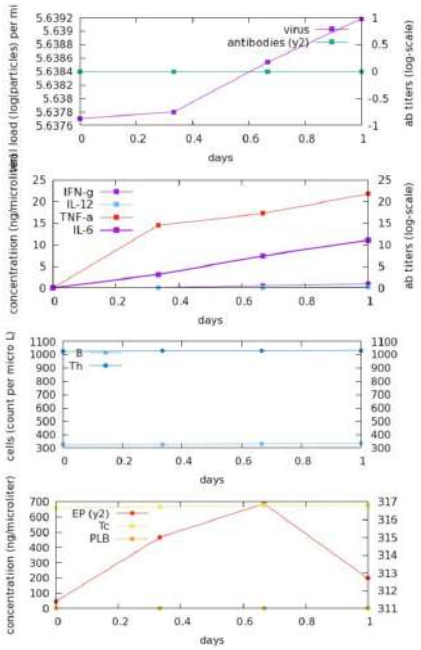
- The SARS-CoV-2's spike molecule affinity to the ACE2 receptor on target cells

$$p_A = u \qquad u \sim U_{[a,b]}$$

$$a = 10^{-3}, b = 10^{-1}$$



disease course



age 0-9
age 10-39
age 40-49
age 50-59
age 60-69
age 70-79
age 80+

1500 infected individuals per age class

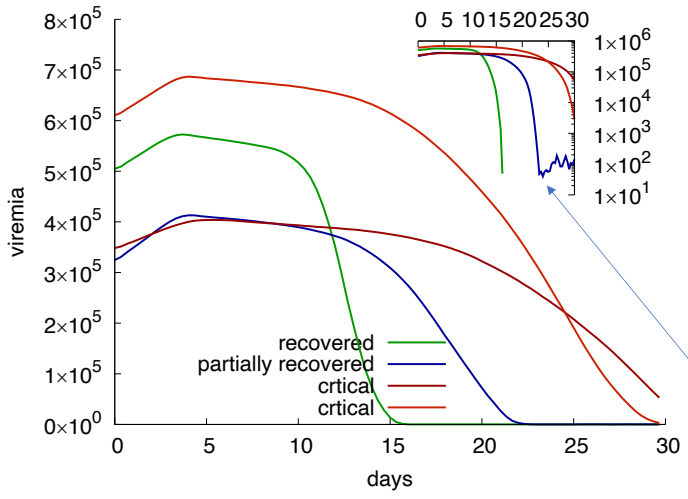
30 days

Initial condition

$IC(age) + (V_0, p_A)$

$IC \to 0 \Longrightarrow$ no immune response
$IC \to 1 \Longrightarrow$ majority of recoveries
$V_0 \to \infty \Longrightarrow$ no recovery
$p_A \to 0 \Longrightarrow$ no infection

case stratification



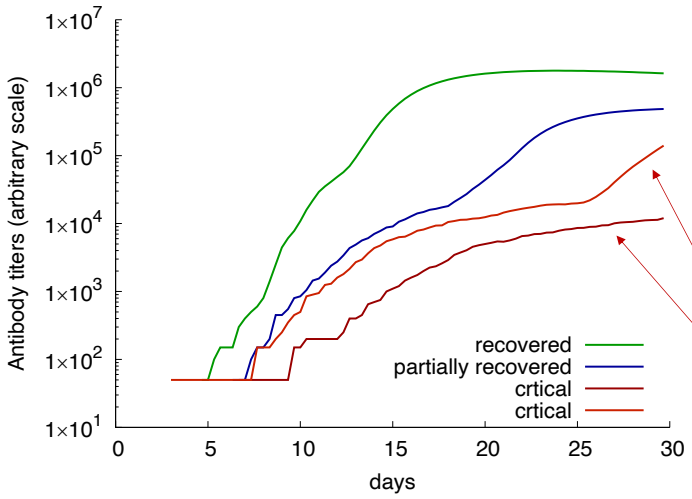
CRITICAL $\Leftrightarrow V_{30} > \theta$

PARTIALLY RECOVERED: $\Leftrightarrow 0 < V_{30} < \theta$

FULLY RECOVERED $\Leftrightarrow V_{30} = 0$

Partially recovered $\supset$ {asymptomatic}

case stratification



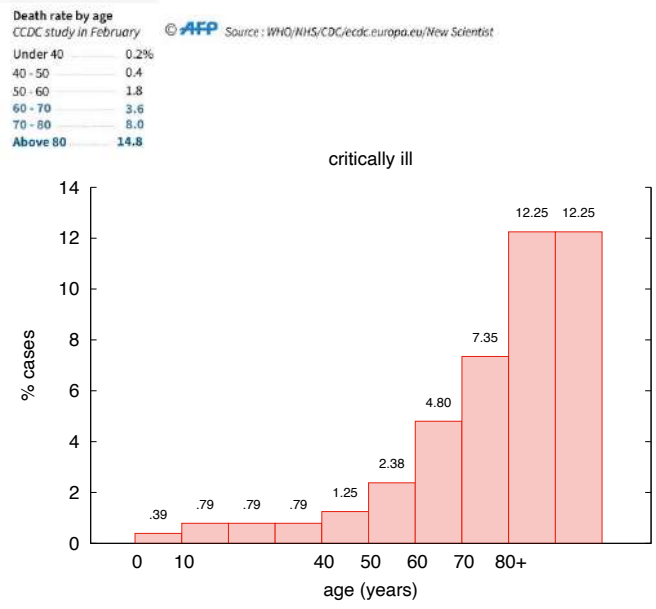
CRITICAL $\Leftrightarrow V_{30} > \theta$

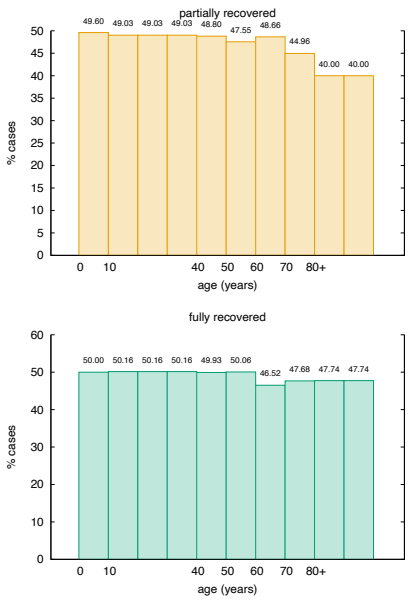
PARTIALLY RECOVERED: $\Leftrightarrow 0 < V_{30} < \theta$

FULLY RECOVERED $\Leftrightarrow V_{30} = 0$

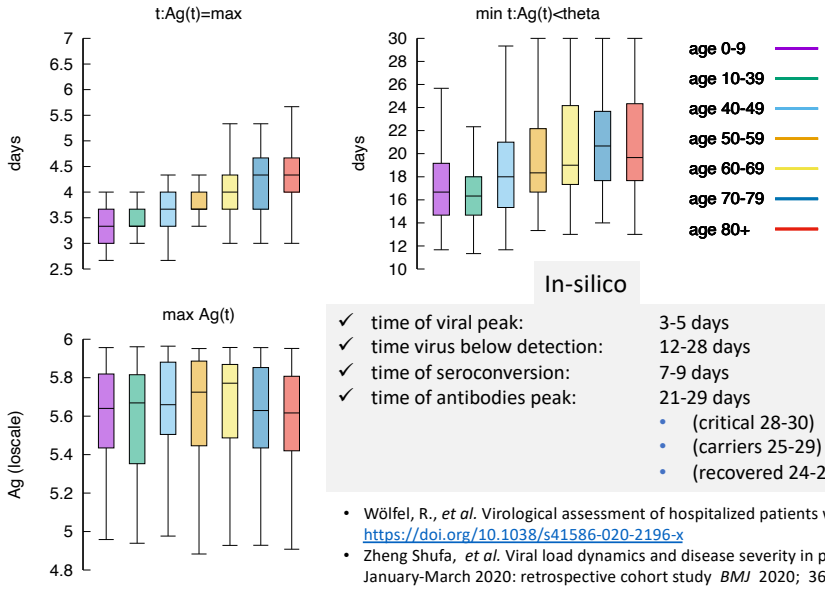
Critical ≡ {late responders} ∪ {weak responders}

virtual cohort parameters' estimation





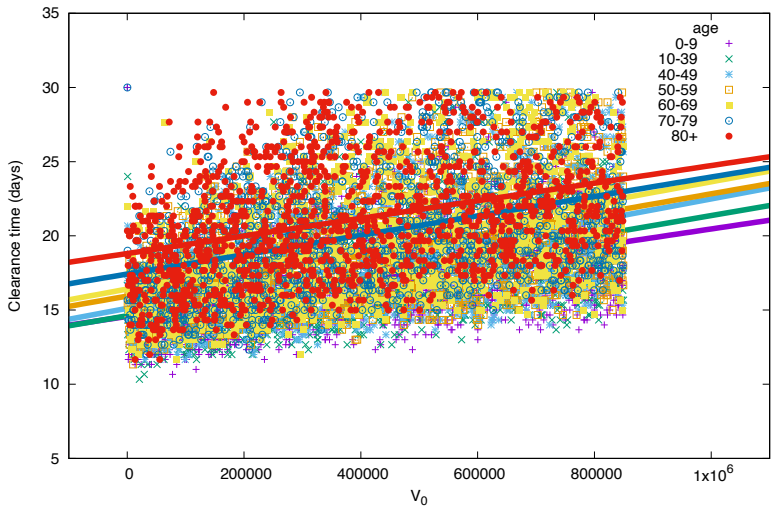
match to reality



In-silico		In-vivo	
✓ time of viral peak:	3-5 days	➢ (pharyngeal swab):	4 days
✓ time virus below detection:	12-28 days	➢ time to recovery:	~20 days
✓ time of seroconversion:	7-9 days	➢ time of seroconversion:	6-12 days
✓ time of antibodies peak:	21-29 days	➢ time of antibodies peak:	16-39 days
• (critical 28-30)		• (critical 22-39)	
• (carriers 25-29)		• (mild 16-21)	
• (recovered 24-29)			

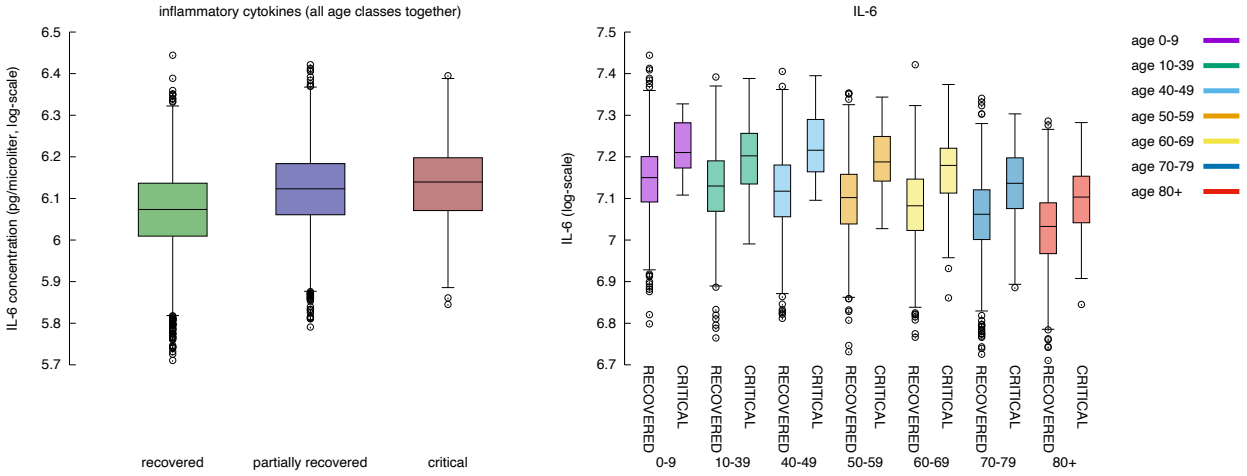
- Wölfel, R., *et al.* Virological assessment of hospitalized patients with COVID-2019. *Nature* 581, 465–469 (2020). https://doi.org/10.1038/s41586-020-2196-x
- Zheng Shufa, *et al.* Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study *BMJ* 2020; 369 :m1443
- Juanjuan Zhao, *et al.*, Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019, *Clinical Infectious Diseases*, , ciaa344, https://doi.org/10.1093/cid/ciaa344

V_0 correlates with disease progression (severity)



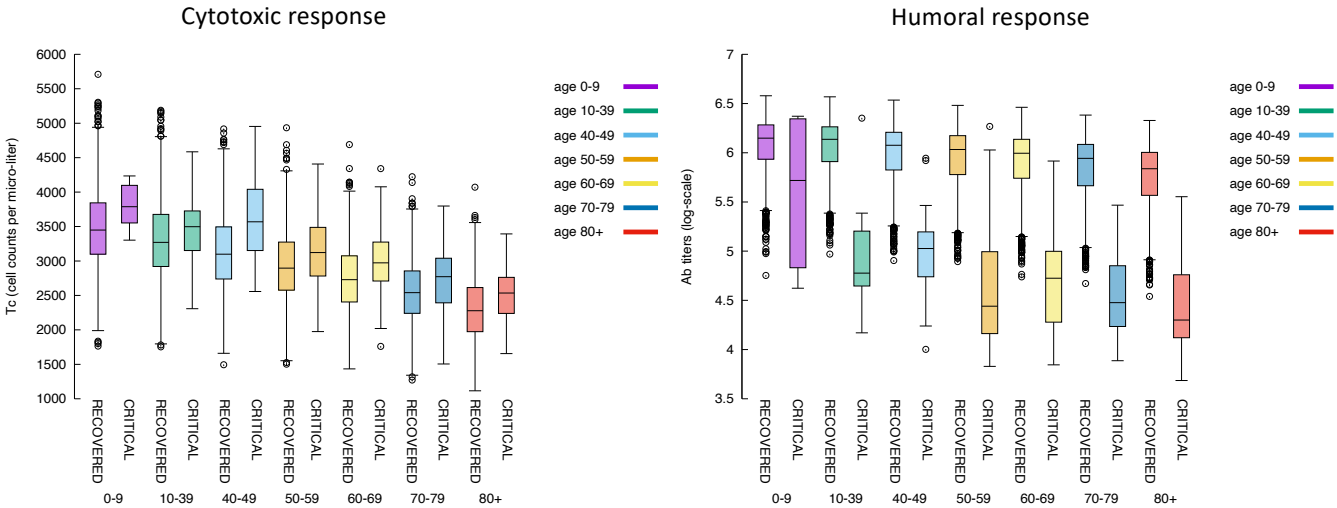
Yu X, Sun S, Shi Y, Wang H, Zhao R, Sheng J. SARS-CoV-2 viral load in sputum correlates with risk of COVID-19 progression. Crit Care. 2020 Apr 23;24(1):170. doi: 10.1186/s13054-020-02893-8. PMID: 32326952; PMCID: PMC7179376.

IL-6 correlates with disease severity



Tobias Herold, Vindi Jurinovic, Chiara Arnreich, Johannes C Hellmuth, Michael von Bergwelt-Baildon, Matthias Klein, Tobias Weinberger. Level of IL-6 predicts respiratory failure in hospitalized symptomatic COVID-19 patients. medRxiv 2020.04.01.20047381; doi:https://doi.org/10.1101/2020.04.01.20047381. *Journal of Allergy and Clinical Immunology (in press)* doi: 10.1016/j.jaci.2020.05.008

humoral response is key for clinical outcome



- https://www.ecdc.europa.eu/en/covid-19/latest-evidence/immune-responses
- To KK, Tsang OT, Leung WS, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis*. 2020;20(5):565-574. doi:10.1016/S1473-3099(20)30196-1

diagnostic value of antibodies

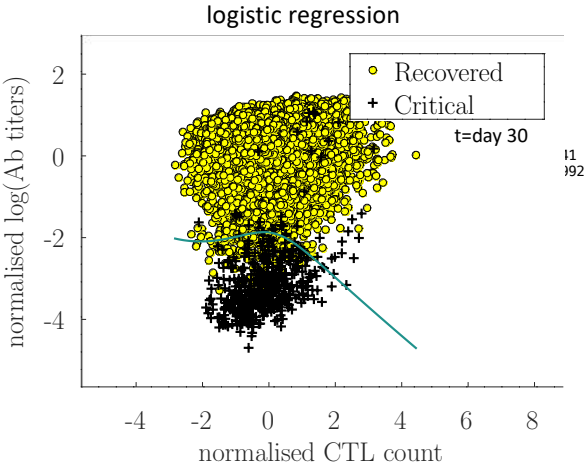
- Understanding the timing of antibody production and seroconversion is key.
  - Determining the optimal time-points for the collection of patient specimens increases the efficacy of diagnostic antibody testing.
  - Knowledge of timing informs the choice of when to obtain peripheral B cells for the development of monoclonal antibody therapeutics.

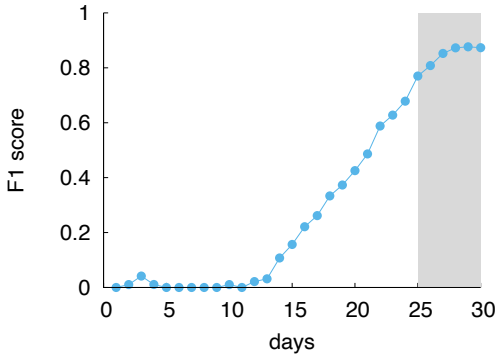
- When is the level of CTLs and Abs informative of the final outcome?
  - classification problem with features

$$(x_1, x_2) = (CTL, Ab)_{t=30} \text{ and target } y \in \{0, 1\}$$ 0=RECOVERED 1=CRITICAL

diagnostic value of antibodies

The optimal time-point for antibody testing is after day 25 from infection, i.e., measurements made before day 25 are not informative for discriminating the disease outcome





$$F1 = 2 \cdot \frac{precision \cdot recall}{precision + recall}$$

Evaluating vaccination protocols

In silico

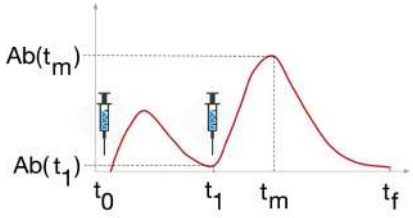
Stolfi P, Castiglione F, Mastrostefano E, Di Biase I, Di Biase S, Palmieri G and Prisco A (2022) In-silico evaluation of adenoviral COVID-19 vaccination protocols: Assessment of immunological memory up to 6 months after the third dose. Front. Immunol. 13:998262. doi: 10.3389/fimmu.2022.998262

evaluating vaccination protocols

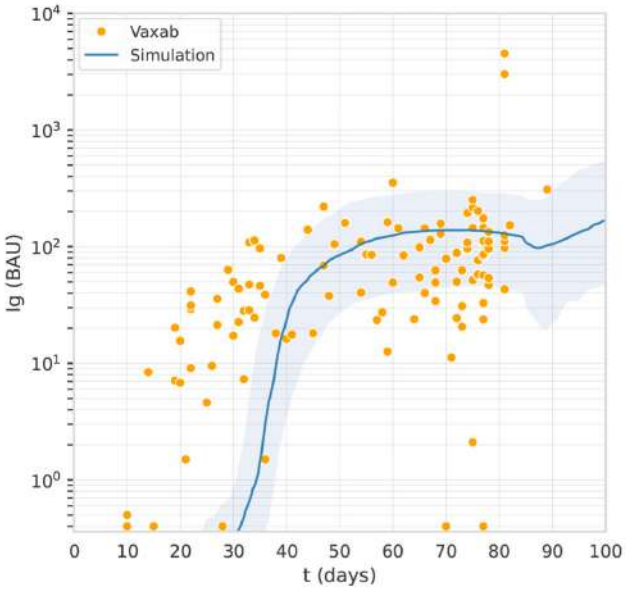
Background: The immune response to adenoviral COVID-19 vaccines is affected by the interval between doses. The optimal interval is unknown.

Aim: We aim to explore in-silico the effect of the interval between vaccine administrations on immunogenicity and to analyze the contribution of pre-existing levels of antibodies, plasma cells, and memory B and T lymphocytes.

Methods: We used a stochastic agent-based immune simulation platform to simulate two-dose and three-dose vaccination protocols with an adenoviral vaccine.
We assessed the immunological memory up to 6 months after the third dose in an adenoviral COVID-19 vaccination protocol



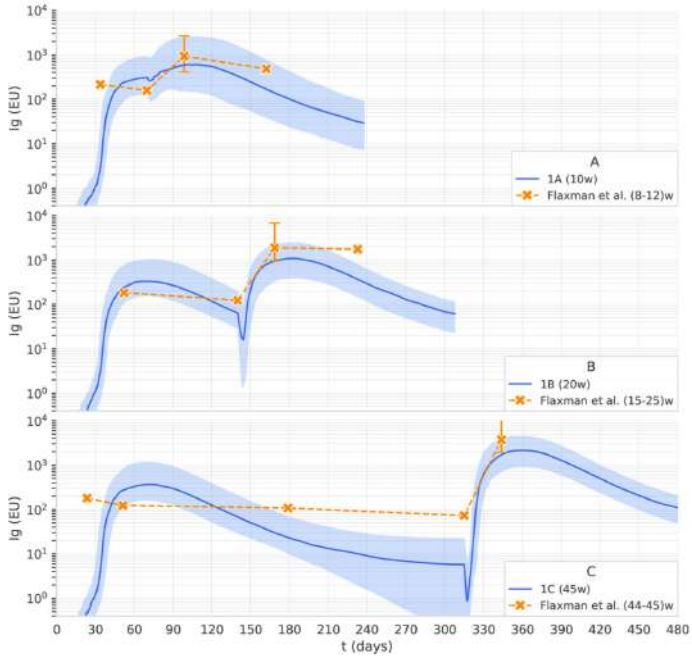
parameters' determination



Methods: We identified the model's parameters fitting anti-Spike antibody levels from individuals immunized with the COVID-19 vaccine AstraZeneca (ChAdOx1-S, Vaxzevria).

The computational model captures the antibody titer trajectory, characterized by a plateau. Overlay of a line graph representing in-silico Ig levels after the first dose (the line represents the median, the shading represents the IQR) with a dot plot representing RBD-Spike Ig BAU in individuals who have received one dose of AstraZeneca, in the Vaxab dataset.

parameters' determination



Methods: We identified the model's parameters fitting anti-Spike antibody levels from individuals immunized with the COVID-19 vaccine AstraZeneca (ChAdOx1-S, Vaxzevria).

The computational model reproduces the effect of the dosing interval observed in clinical trials. Overlay of in-silico Ig levels in two-dose protocols 1A, 1B, and 1C (the line represents the median, the shading represents the IQR) with a dotplot representing median anti-Spike Elisa Units in clinical trial data from Flaxman et al. (2021), corresponding to inter-dose periods of 8-12, 15-25 and 44-45 weeks for panel (A–C), respectively.

in-silico vaccination experiments

	Vaccination regimen	Treatment group (dosing protocol)	Interval between 1st and 2nd dose	Interval between 2nd and 3rd dose	Follow-up
Experiment 1	Two doses	1A	10 weeks	–	6 months after 2nd dose
		1B	20 weeks	–	
		1C	45 weeks	–	
Experiment 2	Three doses	2A	12 weeks	4 months	6 months after 3rd dose
		2B	12 weeks	6 months	
		2C	12 weeks	8 months	
		2D	12 weeks	10 months	
		2E	12 weeks	12 months	
		2F	12 weeks	14 months	
		2G	12 weeks	16 months	
		2H	12 weeks	20 months	
		2I	12 weeks	24 months	

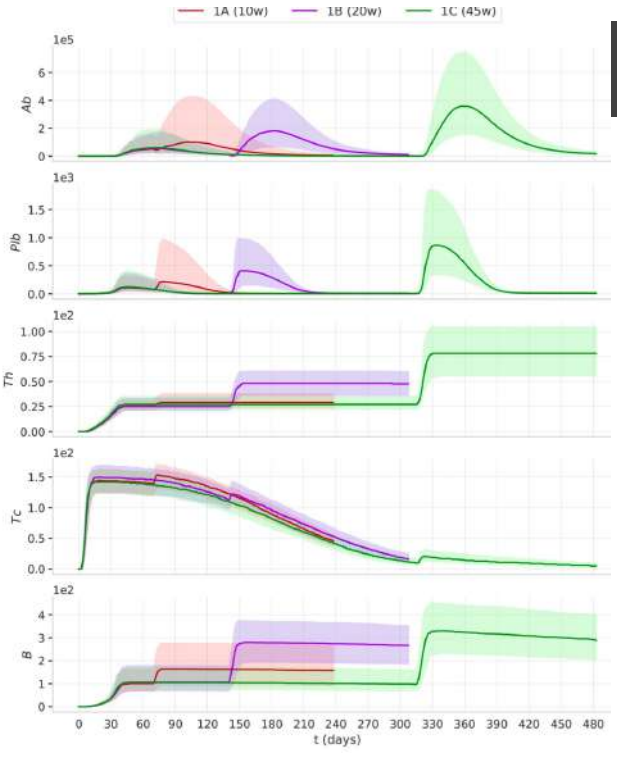
Experiment 1

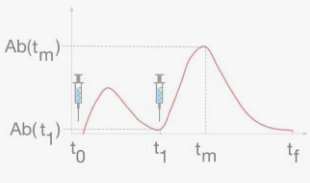
2 injections: Delay = Protocol

- 10 weeks 1A
- 20 weeks 1B
- 45 weeks 1C

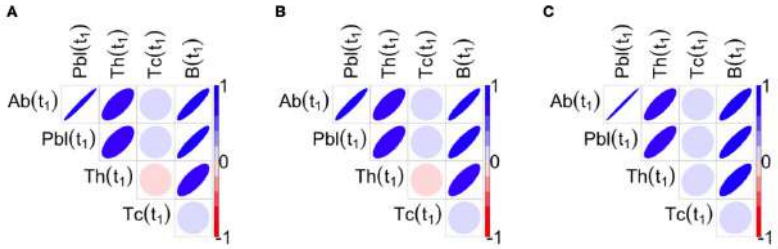
The timing of the second dose affects the dynamics of the immune response. The plots represent the median (solid lines) and IQR (shaded area) of Ab, Plb, Th, Tc, B.

Protocols with longer intervals between the first and second dose achieve higher antibody responses.

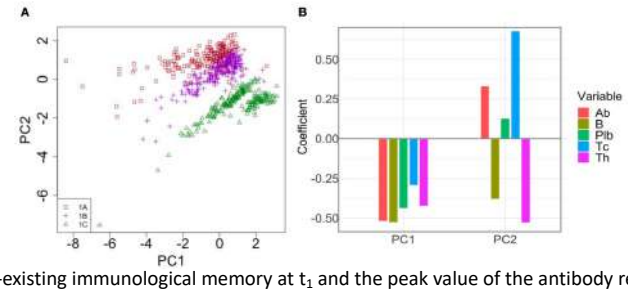




t_1 is the timepoint before the last dose of vaccine



Correlations between the variables of interest at t_1 for the three protocols 1A, 1B, 1C are shown respectively in panels (A–C). Blue ellipses mean positive correlations while red ellipses mean negative correlations. The shape of ellipse helps in the understanding: the more stretched the ellipse the higher the value of the correlation in absolute value. At t_1, antibodies, plasma cells, memory B cells and memory T helper cells are positively correlated among them, whereas Tc is not significantly correlated with the other variables.

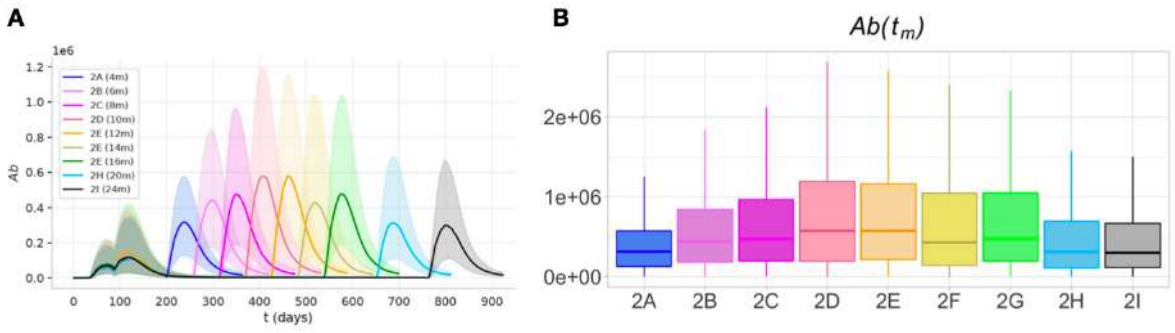


Principal Component Analysis of the correlation between pre-existing immunological memory at t_1 and the peak value of the antibody response to the second dose. (A) The dot plot shows PC1 and PC2 in individuals in treatment groups 1A, 1B and 1C. PC2 separates the different dosing protocols. (B) Loadings of PC1 and PC2. In PC2, Tc has the highest loading.

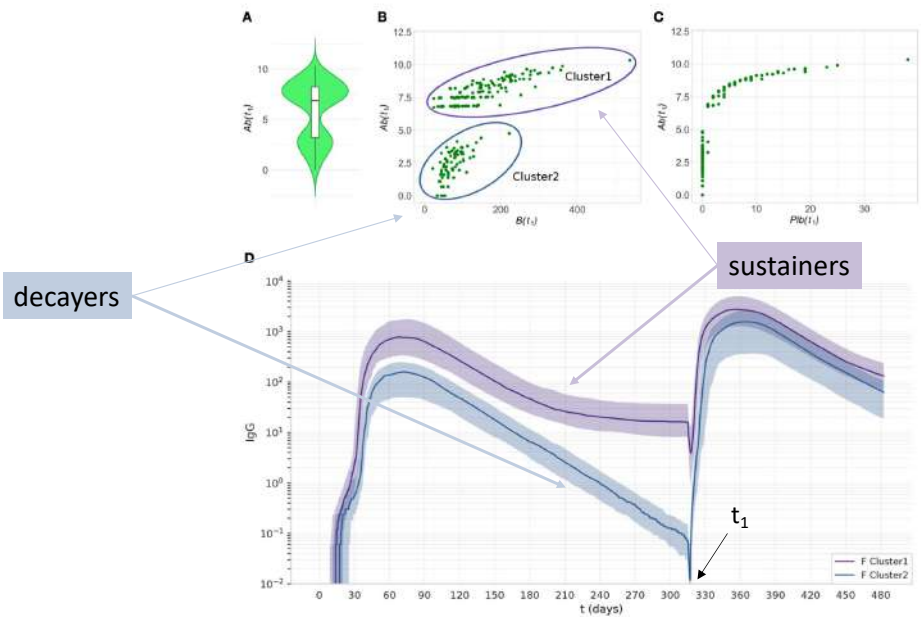
Experiment 2

3 injections: Delay between 2nd and 3rd dose

✓ 4 to 24 months



The optimal immunogenicity of antibody response to the third dose is achieved over a large time window. The protocols with intervals between the second and third dose between 6 and 16 months achieve peak antibody responses significantly higher ($p < 0.05$)) than shorter or longer protocols. (A) The plots represent the dynamics of the median (solid lines) and the IQR (shaded area) of variable Ab, in experiments 2A-I. (B) The box plots show the median, IQR, and range of the antibody peak after the third dose, $Ab(t_m)$.



The two clusters identified by PCA can be separated by their level of Ab(t_1) Data from experiment 1C are reported. (A) The violin plot of Ab(t_1) reveals two clusters with different levels of Ab. (B) The scatterplot shows that the individuals with low antibody levels have, in most cases, no plasma cells. (C) the scatterplot shows that the individuals with low levels of antibody have memory B cells. (D) The antibody dynamics of the two clusters is different, cluster 1 represents antibody **sustainers**, and cluster 2 represents antibody **decayers**. The plots represent the median (lines) and IQR (shaded area) of variable Ab in cluster 1 and cluster 2.

predictions/conclusions

- optimal immunogenicity of 3rd dose achieved over a large time window (6 to 16 months after 2nd dose)
- strong positive correlation between antibodies, plasma cells, memory B cells, and memory CD4 T cells after the first dose of vaccine ⟹ antibody titer (measured very easily) is a biomarker of memory Bs, PLBs, and CD4 T cells.
- anti-spike cytotoxic T cells (a desired outcome of immunization) can contribute to reduced immunogenicity of subsequent doses.
- memory B cells and memory CD8 T cells have opposite effects on the antibody response to the boost. Increased antibody response to late booster doses appears to be due to the combined effect of the decline in antibody levels and in the number of memory CD8 T cells.

predictions/conclusions

- antibody sustainers have more memory T helper and memory B cells than antibody decayers $\Longrightarrow$ a stronger response of T helper and B cells has a higher probability of resulting in the development of long-lived plasma cells.
- while the time window for the optimal immunogenicity of the third dose of an adenoviral vaccine is ample (6-16 months), antibody decayers may benefit from receiving the third dose at the beginning of the optimal time window, to avoid loss of serological protection.

Thank you for your attention

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